

VIRAL CLONING SYSTEM

Figure 1

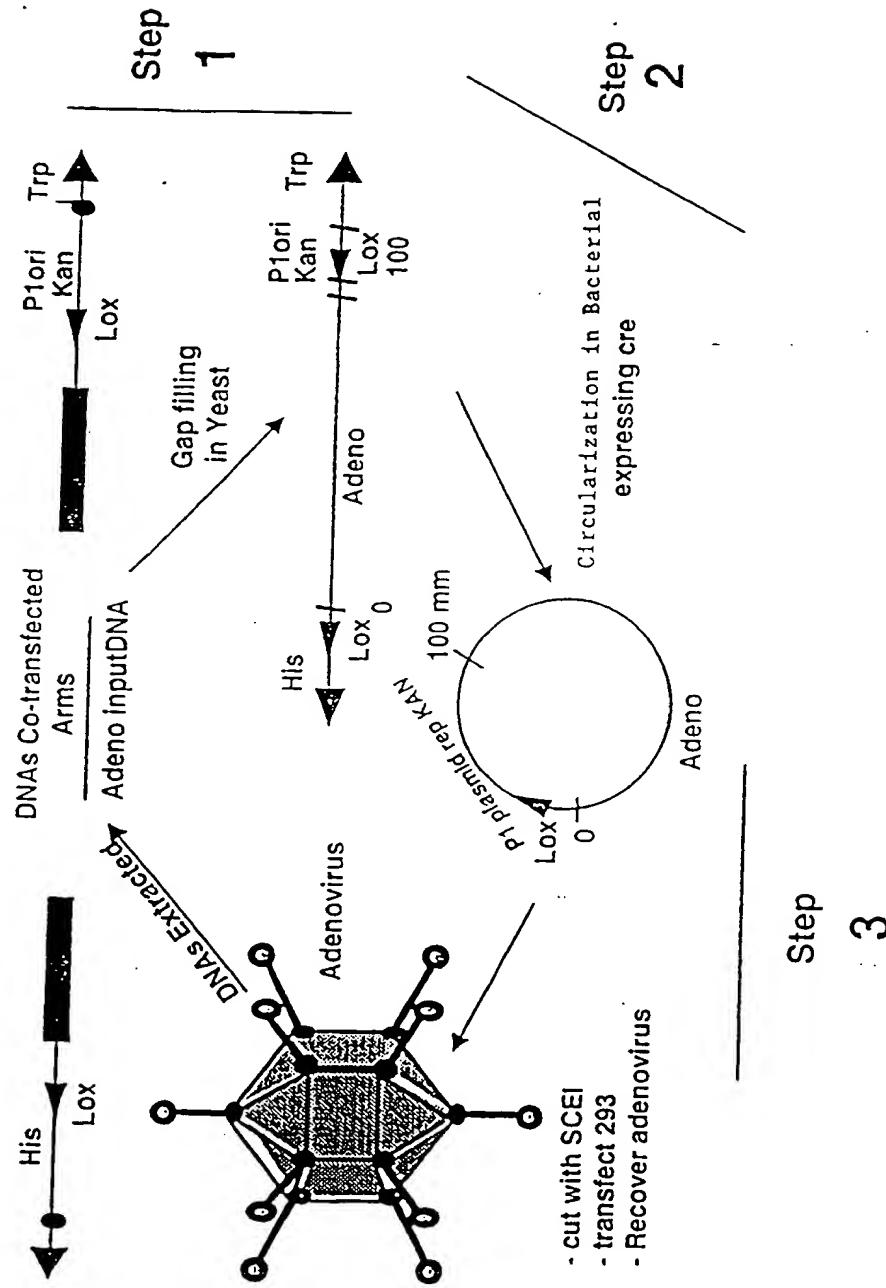
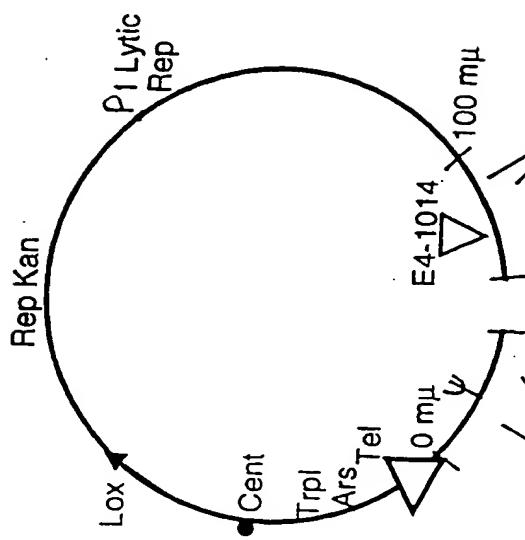


Figure 2

Circular Viral Cloning System

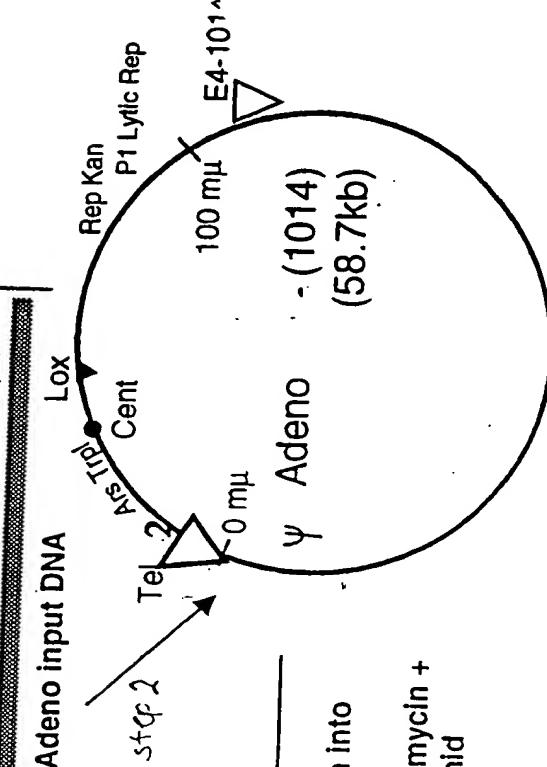


Gap Filling in Yeast

Step 1

Cotransfect with Viral DNA
into Yeast

0 mμ E4-1014 100 mμ



Step 2

Step 3

- electroporation into bacterial strain
- select for Kanamycin +
- Recover Plasmid

- cut with I-SceI
- transfect 293
- Recover adenovirus

Adenovirus

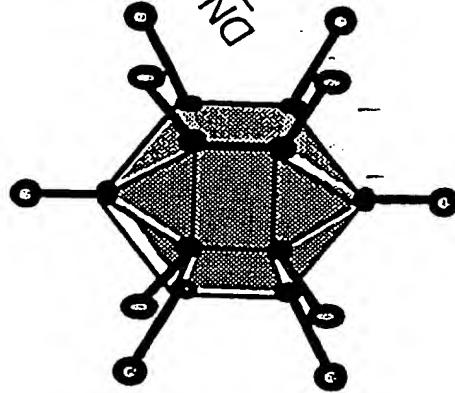


Figure 3

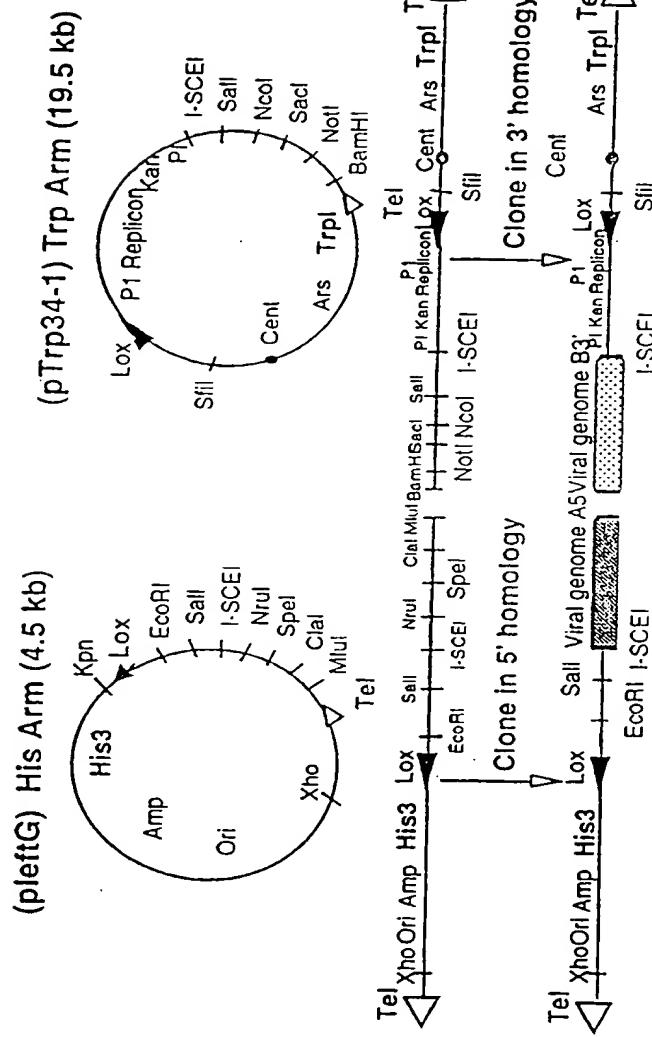


Figure 4

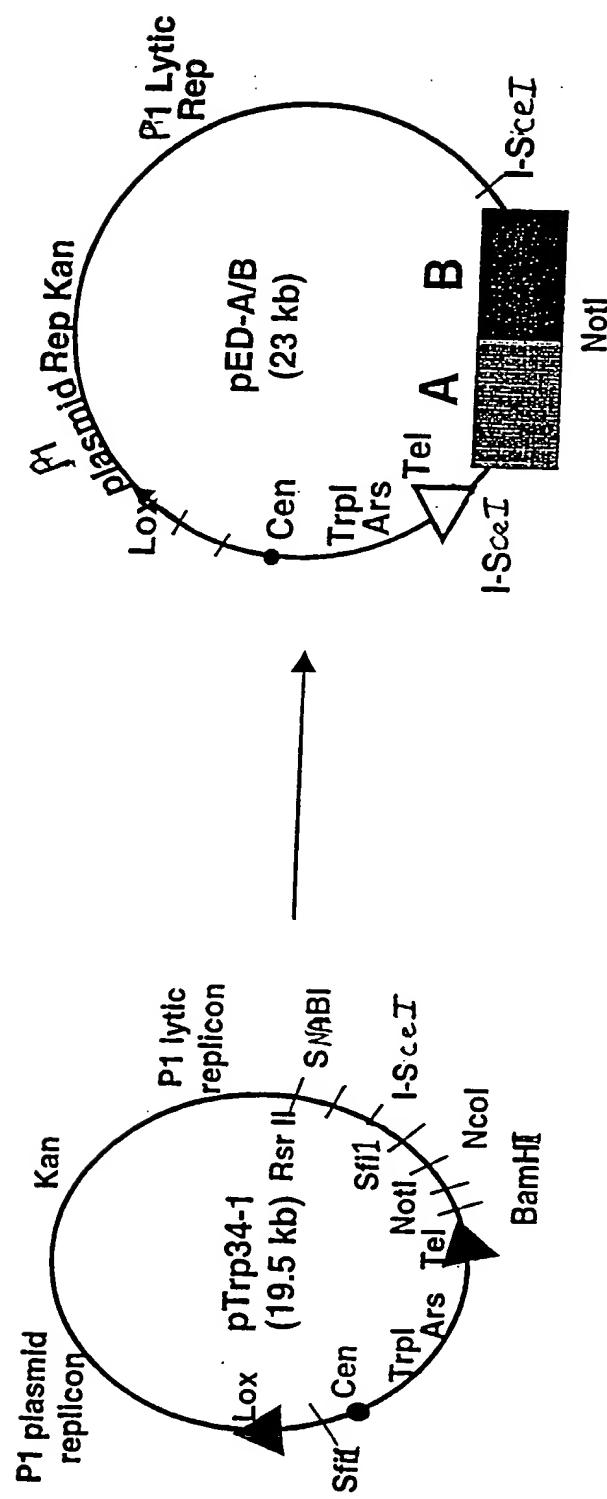


Figure 5 His Arm Construction

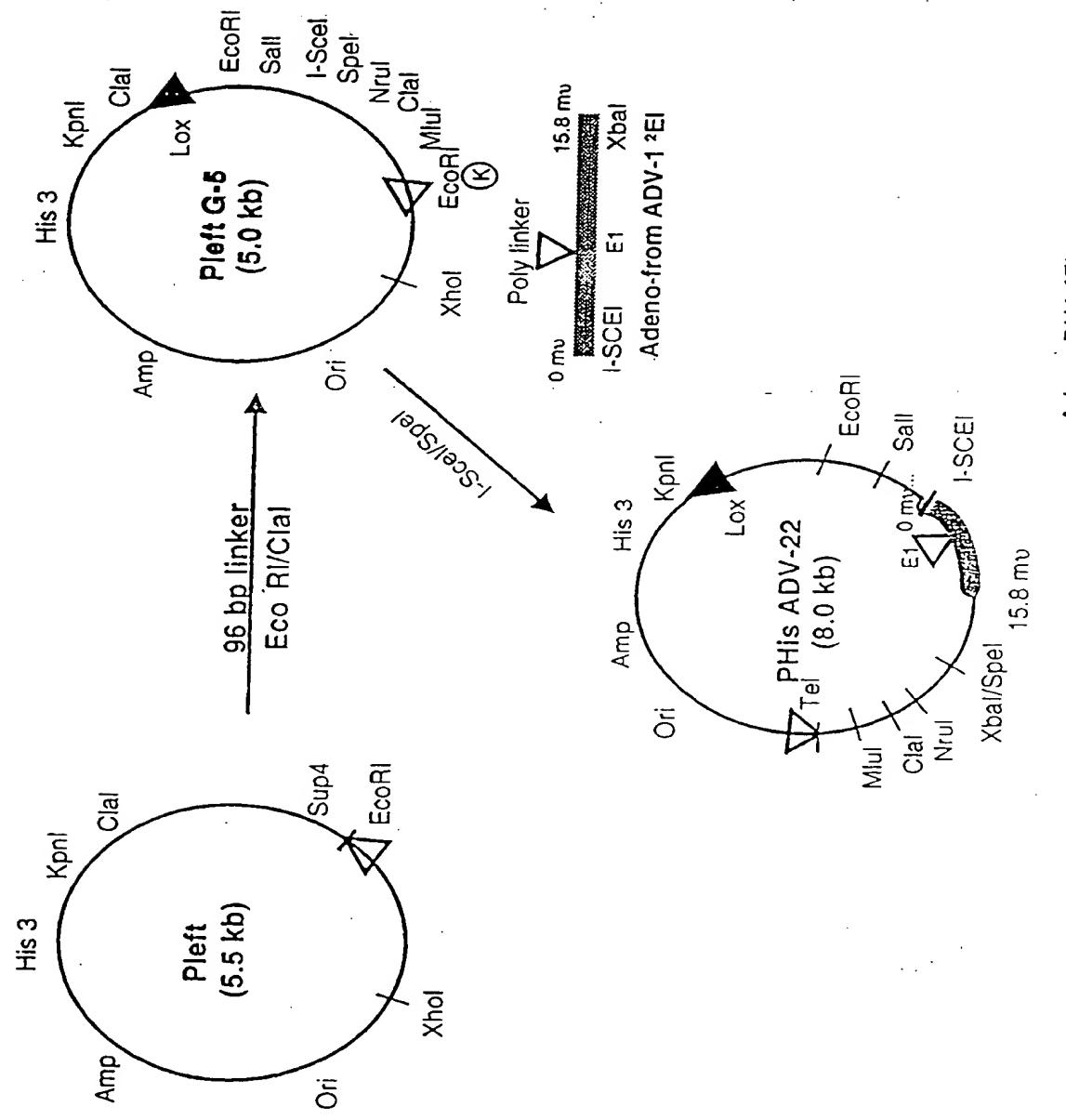


Figure 6 Trp Arm Construction

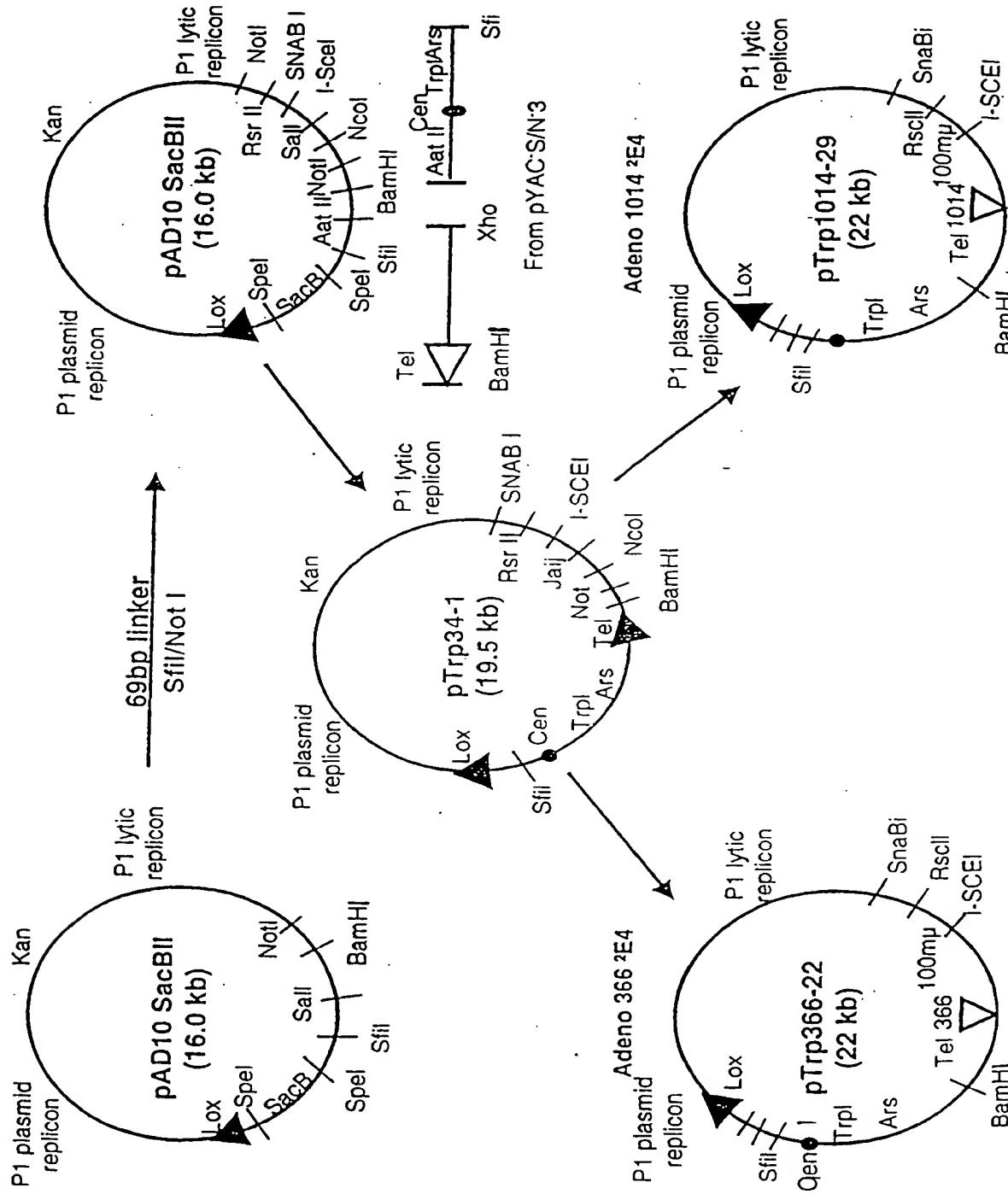


Figure 7

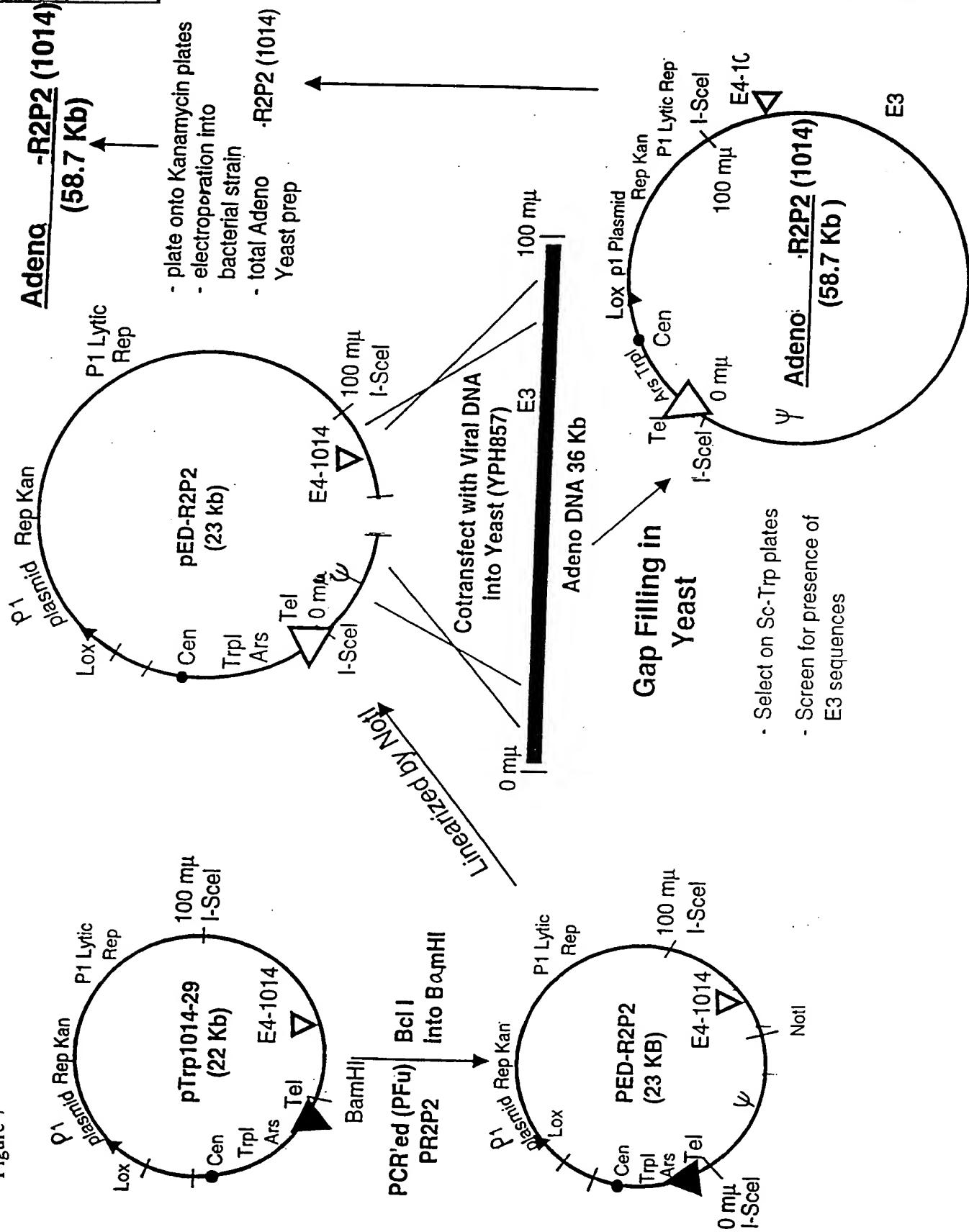


Figure 8

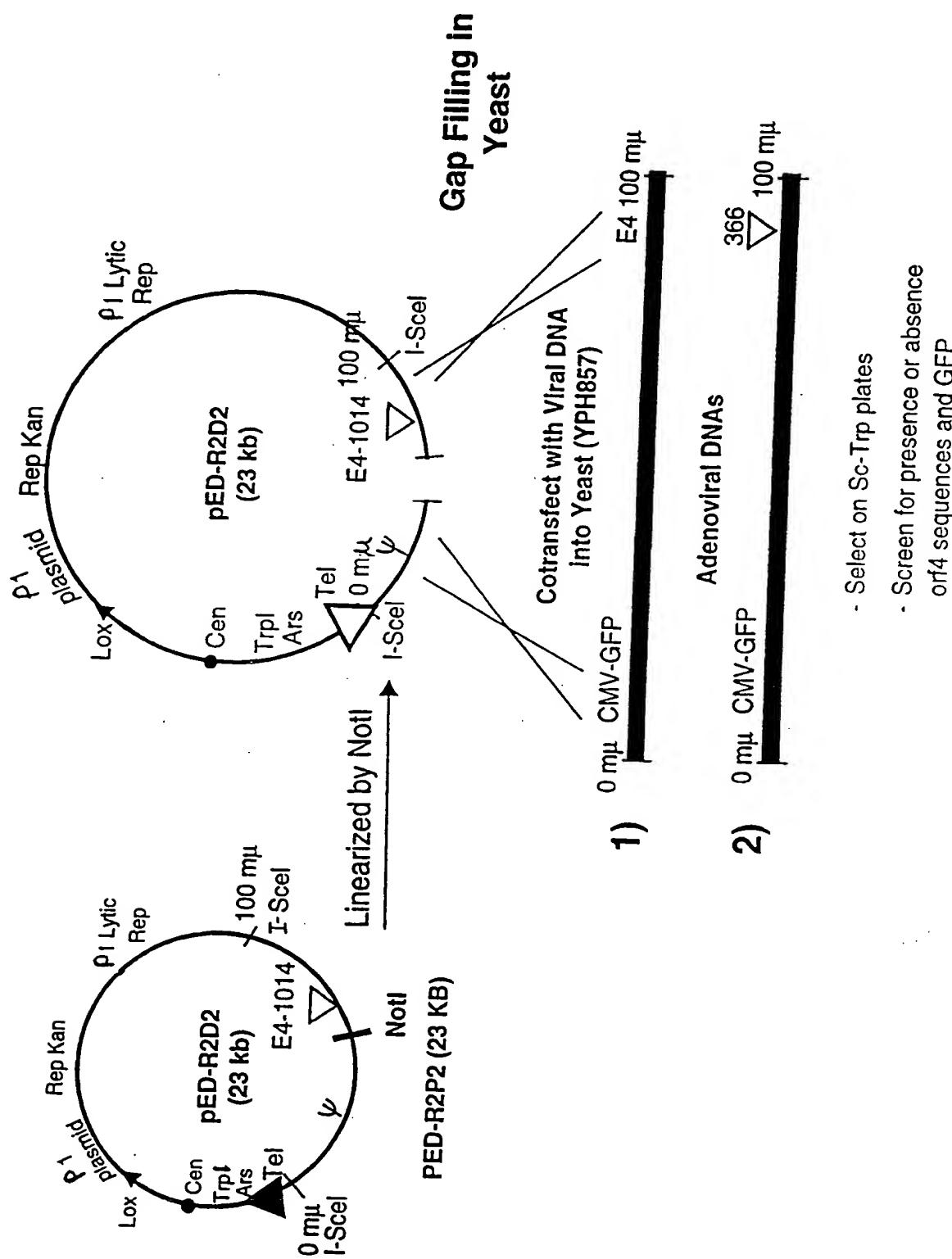


Figure 9

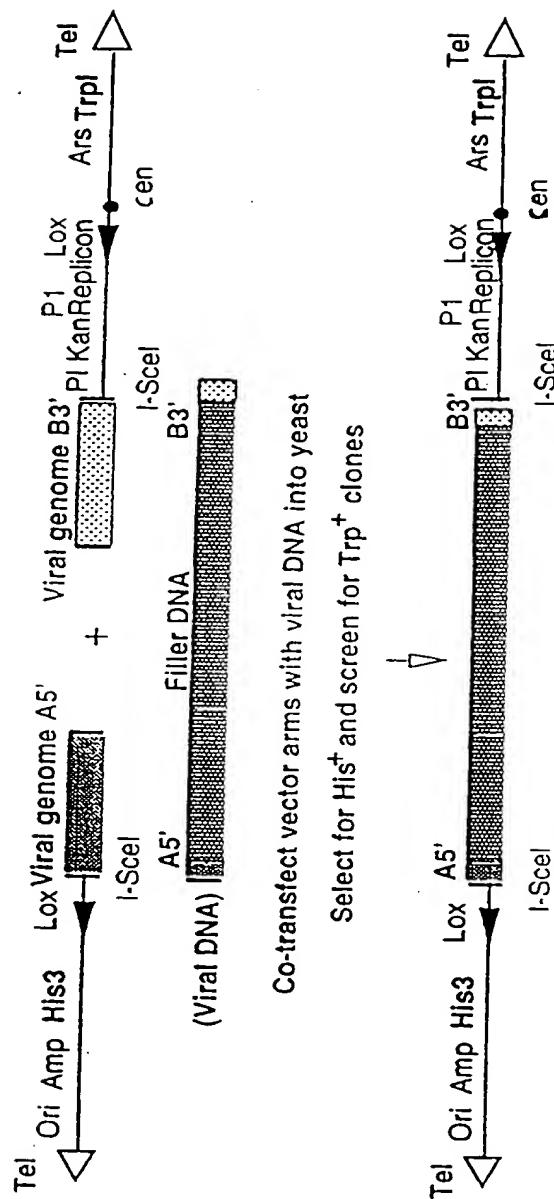


Figure 10 Lys replacement arm

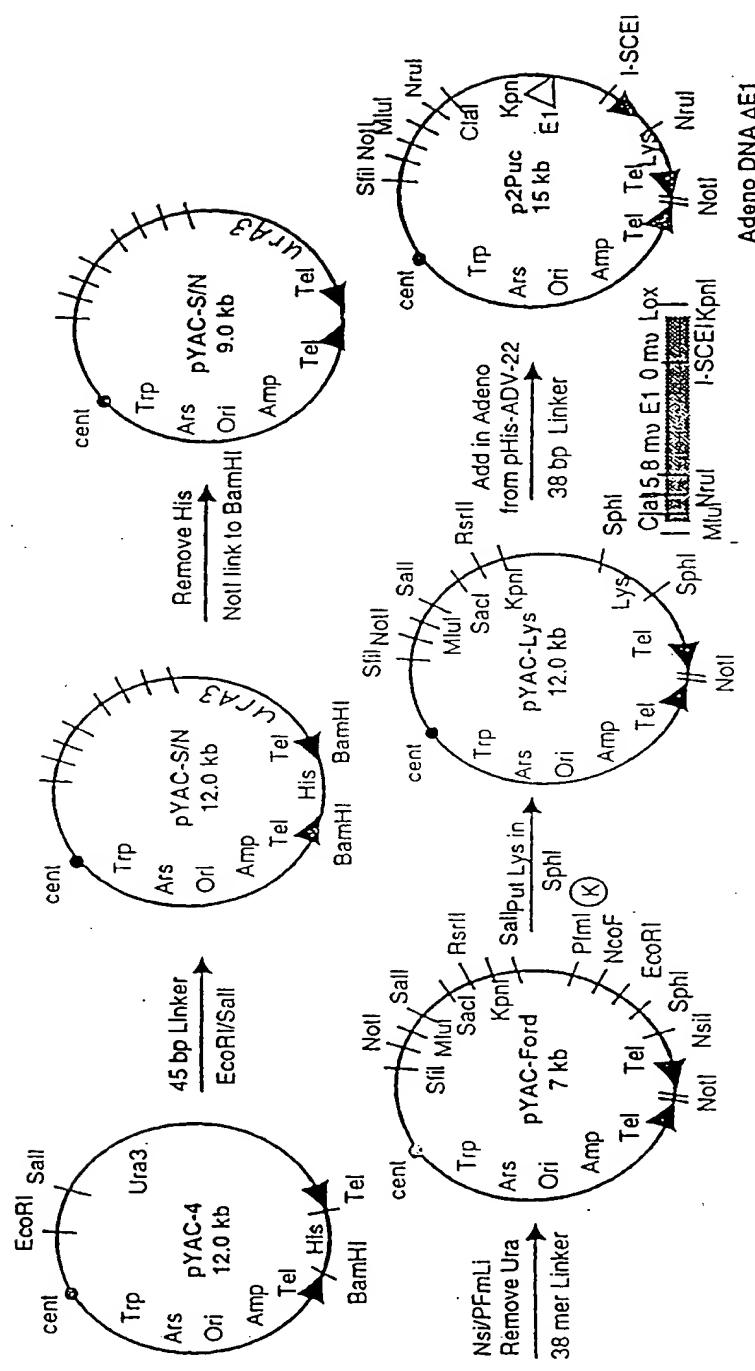
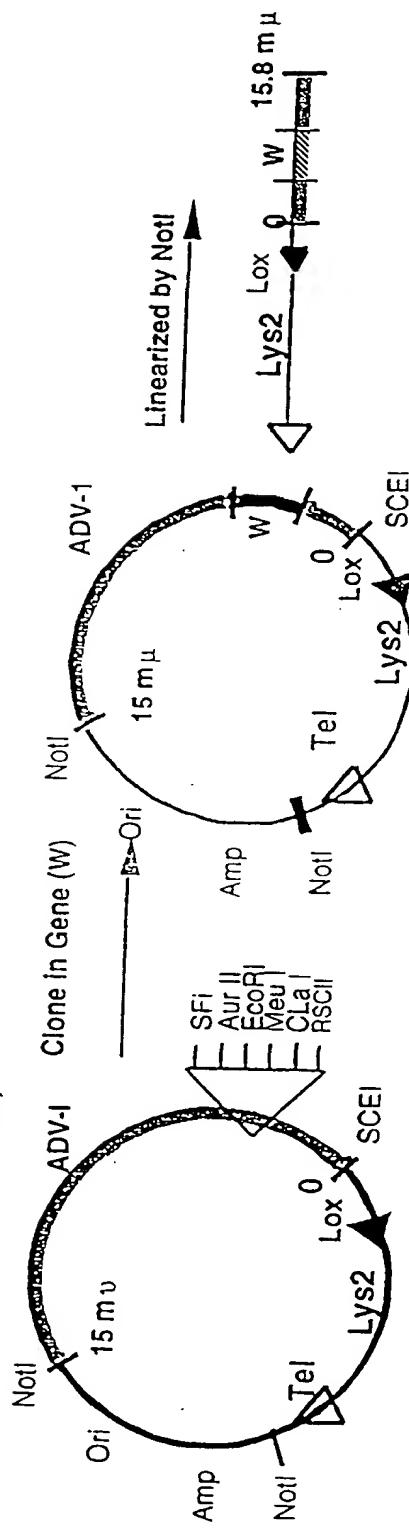


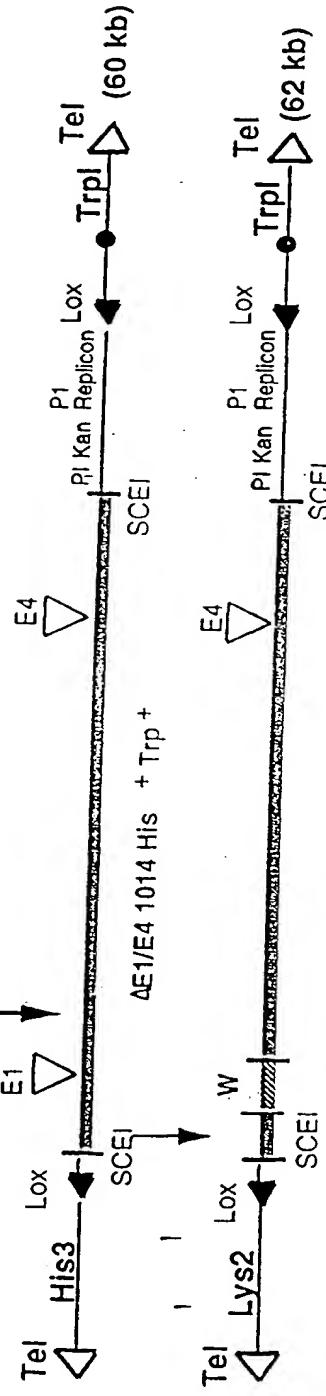
Figure 11

Viral Modifications: Gene Arm Replacement

Lys replacement arm (12.0kb)

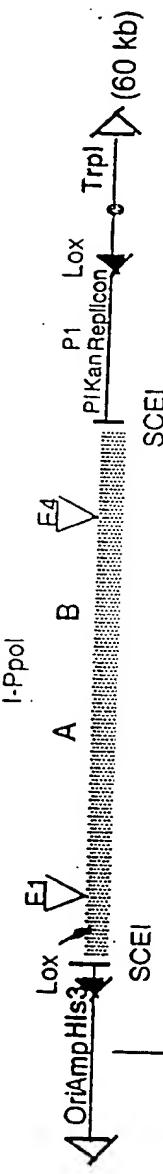


Tel Lys2 Lox 0 W 15.8 mμ • Transfect linearized vector into yeast strain Y $\Delta E1/E4$ 1014
 • Selection is done for Lys⁺ and screened for the loss of His

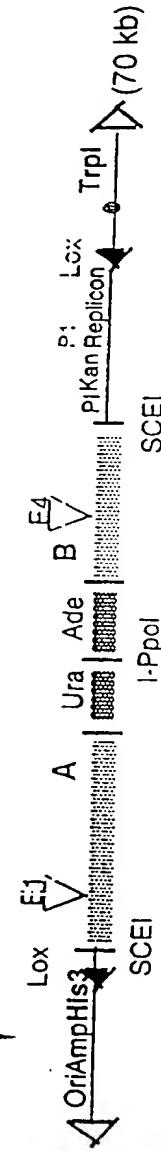


W $\Delta E1/E4$ 1014 Lys⁺ Trp⁺ His⁻

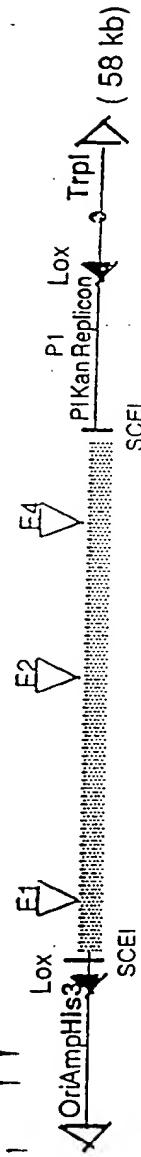
Figure 12 Viral Modifications: One Step In



- $\Delta E1/E4$ 1014 is transformed with Ura/Ade omega vector -
- which contains 2 points of homologous sequences A and B, PCR'd out of ADV-5



- $\Delta E1/E4$ (His⁺Trp⁺Ura⁺Ade⁺) is transformed with Adeno 902 DNA.
- Clones are selected on SC⁺ 5FO⁺ His plates and screened for loss of Ade (red), Ura with the presence of Trp



$\Delta E1/E2/E4$ 1014 His+trp Ade(red) Ura

Figure 13

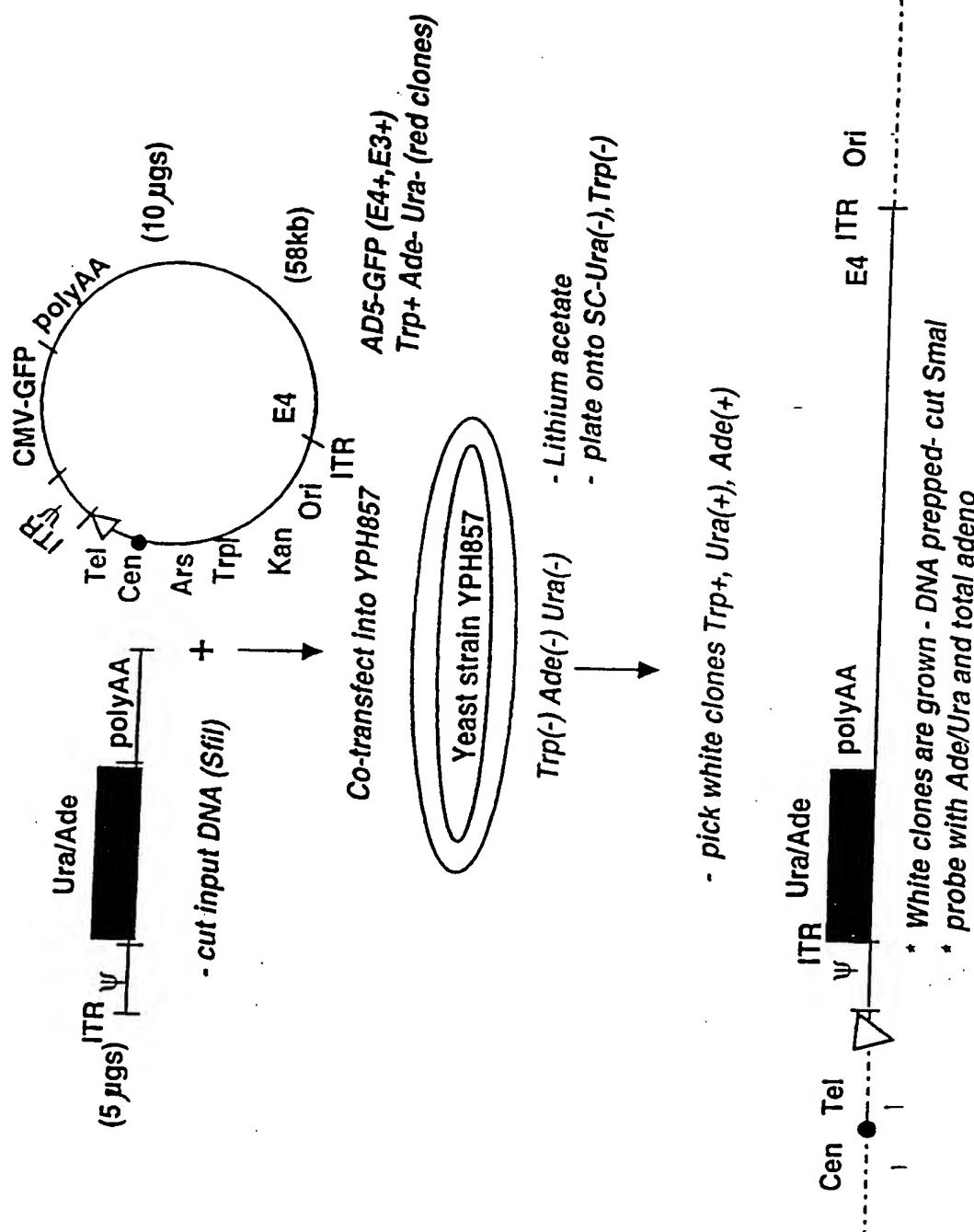


Figure 14 Viral Modifications: Two Step In and Out

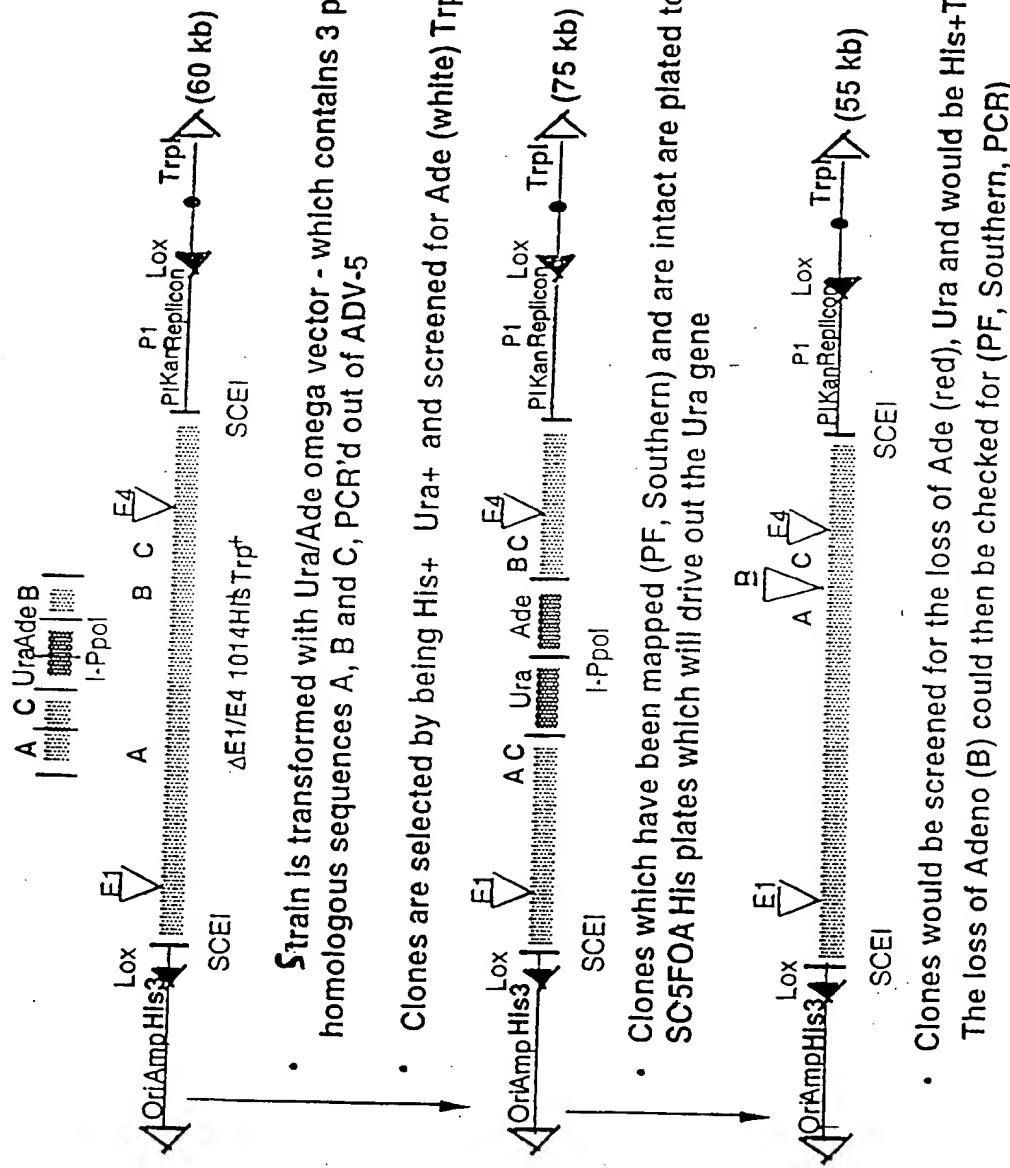
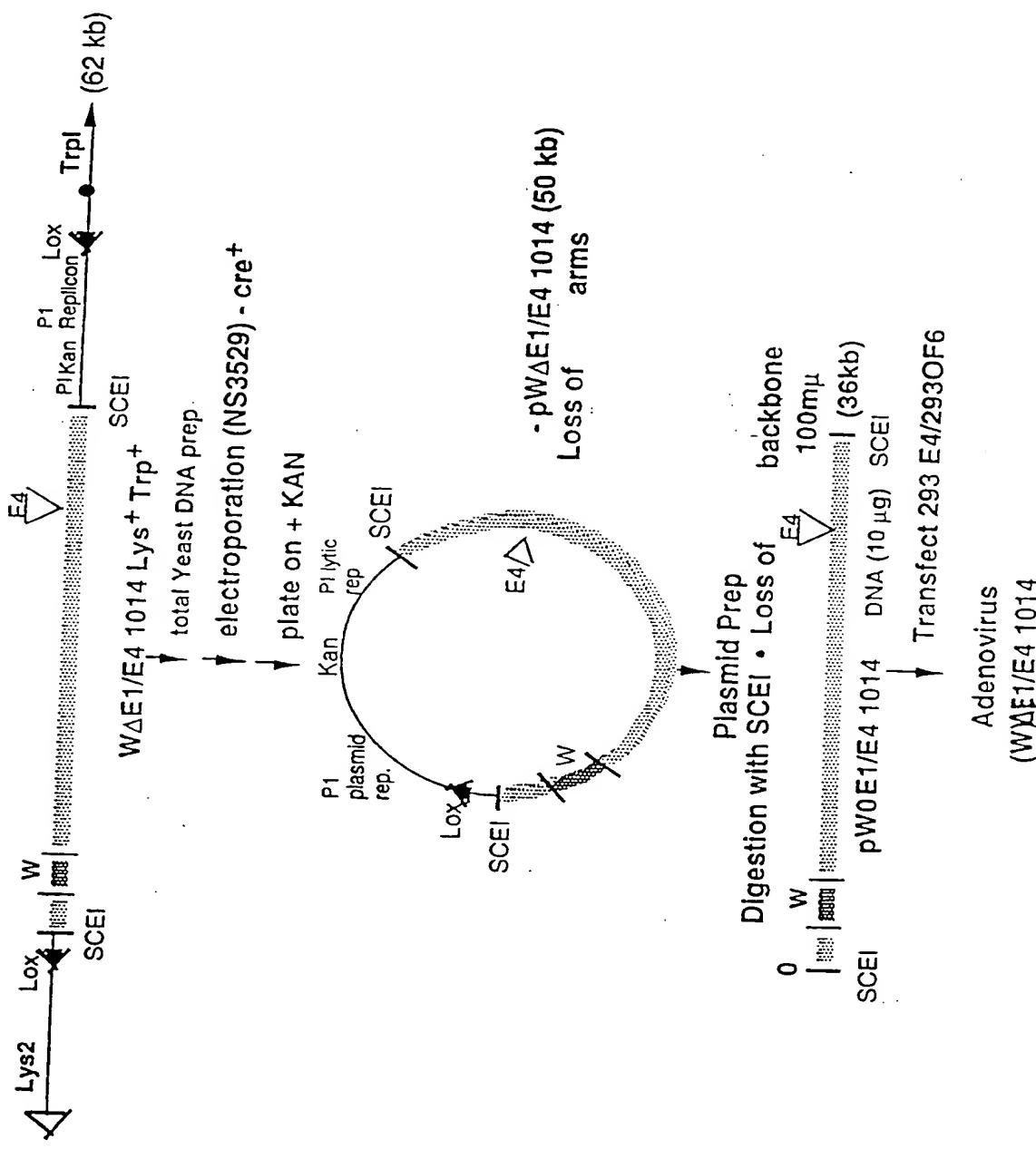


Figure 15



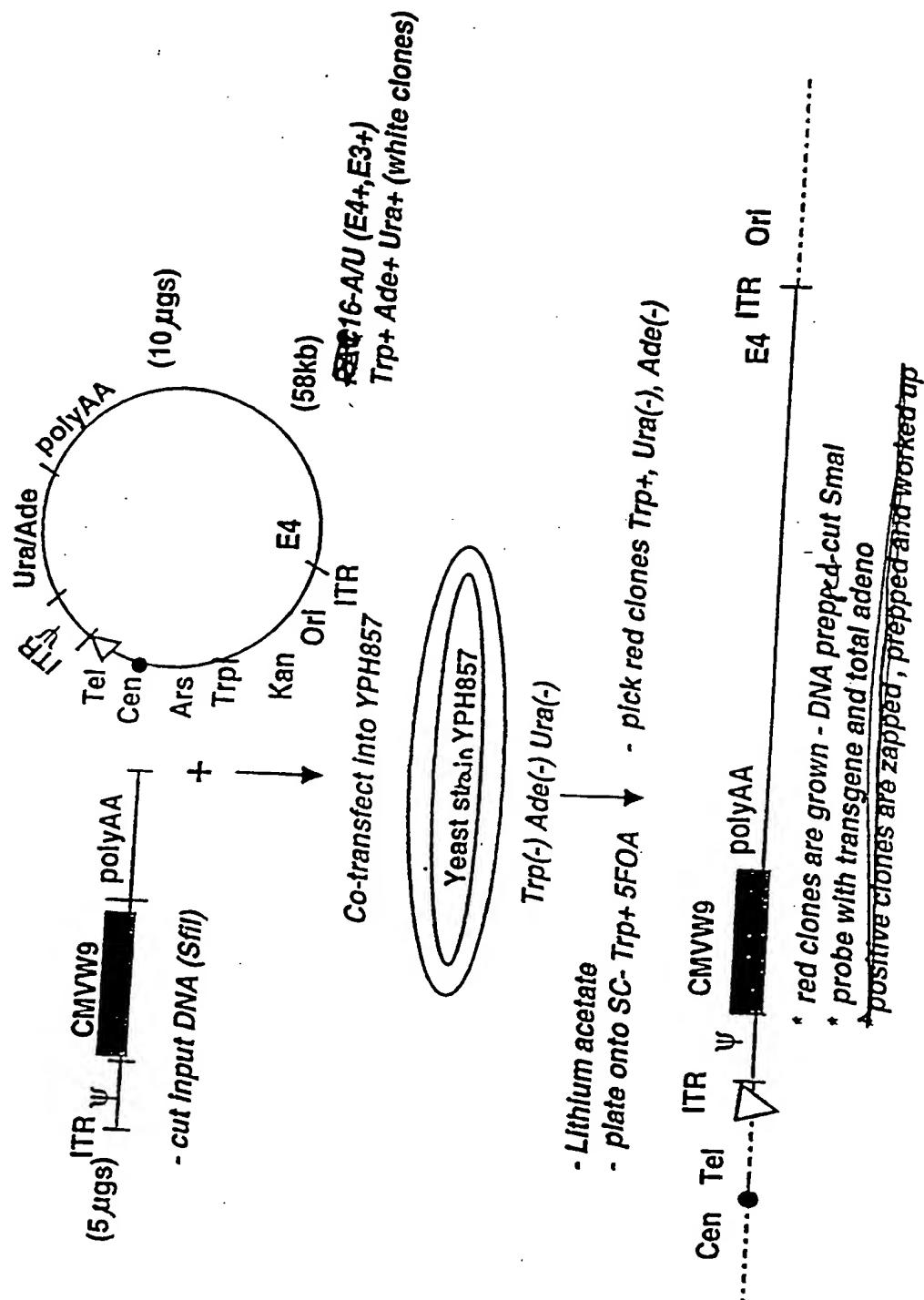


Figure 16